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HEMORRHAGIC FEVER WITH RENAL SYNDROME (KOREAN HEMORRHAGIC FEVER)

ANNUAL SUMMARY REPORT

HO WANG LEE, M.D.

June 29, 1990

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Grant No. DAMD17-86-G-6011

Korea University College of Medicine
4, 2nd Street Myungyundong
Chongnoku, Seoul 110-702, Korea

SECURITY CLASSIFICATION OF THIS PAGE

AD-226-463

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; Distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): HFRS-2	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Korea University College of Medicine; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): 4, 2nd Street, Myungyundong, Chongnoku, Seoul 110-702, Korea	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: US Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-86-G-6011
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21702-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M1-62770A071; TASK NO. AB; WORK UNIT ACCESSION NO. 381

11. TITLE (Include Security Classification)
HEMORRHAGIC FEVER WITH RENAL SYNDROME (KOREAN HEMORRHAGIC FEVER)

12. PERSONAL AUTHOR(S)
Lee Ho Wang

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 89/2/10 TO 90/2/9	90/6/29	

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB GROUP	Hantavirus, Hantaan, Seoul, Puumala virus, global survey, hemorrhagic diseases, high density particle agglutination IFAT, PPAT, RA 1
06	03		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

World-wide, about 200,000 people are hospitalized with Hemorrhagic fever with renal syndrome (HFRS) (3-10% fatality) each year. The etiologic agents of HFRS are Hantaan, Seoul and Puumala viruses of the genus Hantavirus, family Bunyaviridae. A severe form of HFRS, caused by Hantaan virus, occurs in Asia and eastern parts of Europe, a moderate form, caused by Seoul virus, occurs in Asia, and a mild form, caused by Puumala virus, occurs in Europe. The reservoirs of hantaviruses are rodents and other small mammals. Global surveys of the distribution of hantaviruses and surveillance of HFRS are important for prevention of this highly fatal disease. A simple and rapid serologic diagnostic test for HFRS in the areas where hantaviruses exist is urgently needed. It is also important to investigate antigenic differences of strains of Hantavirus isolated from rodents caught in non-endemic areas of the world because HFRS patients have never been documented in many areas despite the finding of positive rodents there.

The methods for diagnosis of HFRS, isolation of hantaviruses from man and rodents are

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Virginia Miller	22b. TELEPHONE (Include Area Code): 301/663-7325
	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete — SECURITY CLASSIFICATION OF THIS PAGE

described previously. A new high density silicone particles were used for a rapid serologic diagnostic test for HFRS.

There were 430 cases of HFRS in Korea in 1989 and large outbreaks of scrub typhus, murine typhus, spotted fever and leptospirosis occurred before and during the epidemic season of HFRS. Antibody against hantaviruses was measured within forty minutes by a passive agglutination procedure using high density composite particles coated with purified Hantaan virus antigen. Antigen for the reaction was prepared from the brains of suckling rats infected with Hantaan virus. This method is more sensitive than the immunofluorescent antibody technique and the antigen reacted with antibodies to Hantaan, Seoul and Puumala viruses. Serologic studies of 42 hantaviruses isolated from HFRS patients and from animals throughout the world indicated that there are 6 or 7 serotypes. In the 1990s, it is highly possible to identify HFRS and HFRS-like illnesses caused by hantaviruses in parts of the world where HFRS is not known because of the availability of serodiagnostic tests.

SUMMARY

World-wide, about 200,000 people are hospitalized with Hemorrhagic fever with renal syndrome (HFRS) (3-10% fatality) each year. The etiologic agents of HFRS are Hantaan, Seoul and Puumala viruses of the genus Hantavirus, family Bunyaviridae. A severe form of HFRS, caused by Hantaan virus, occurs in Asia and eastern parts of Europe, a moderate form, caused by Seoul virus, occurs in Asia, and a mild form, caused by Puumala virus, occurs in Europe. Total number of hospitalized HFRS patients serologically confirmed at our laboratory among 1,886 suspect hemorrhagic disease in 1989 in Korea was 430, and 37 and 3 of them were ROK Army and US Army soldiers, respectively. Outbreaks of scrub typhus, murine typhus and spotted fever occurred about a month before and during the epidemic season of HFRS and number of serologically confirmed cases were 358, 359 and 83, respectively. Epidemiologic features of these hemorrhagic diseases in 1989 were almost same as in 1988. A simple and rapid serologic diagnostic test for hantavirus infection was developed by high density particle agglutination. Antibodies against hantaviruses was measured within forty minutes by a passive agglutination procedure using high density composite particles coated with purified Hantaan virus antigen. Antigen for the reaction was prepared from the brains of suckling rats infected with Hantaan virus, using ultracentrifugation, protamine sulfate and ethyl alcohol treatment. This method is more sensitive than the immuno-fluorescent antibody technique and the antigen reacted with antibodies to Hantaan, Seoul and Puumala viruses. Hantaan virus occurs in Asia and in eastern parts of Europe, Seoul-like viruses occur world-wide, Puumala virus occurs in Europe, and Prospect Hill and Leaky viruses have been isolated in U.S.A. The reservoirs of hantaviruses are rodents and other small mammals. Serologic studies of 42 hantaviruses isolated from HFRS patients and from animals indicated that there are 6 or 7 serotypes. In the 1990s, it is highly possible to identify HFRS and HFRS-like illnesses caused by hantaviruses in parts of the world where HFRS is not known because of the availability of serodiagnostic tests.

FOREWORD

In conducting the research described in this report, the investigators (s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

During the Korean War more than 3,200 United Nations troops in Korea developed a rare hemorrhagic fever, a situation that attracted worldwide attention (1). Since then it has been known as Korean hemorrhagic fever (KHF) in Korea. This disease was an important military problem because large epidemics occurred among soldiers during several wars. More than 12,600 cases of epidemic hemorrhagic fever (EHF) occurred among one million Japanese soldiers in Manchuria (2) and several hundred cases among Russian soldiers in the Far East (3) during World War II. Several thousand cases of war nephritis, clinically similar to Nephropathia epidemica (NE), were reported among British soldiers stationed in Flanders during World War I (4), and about 16,000 cases of NE occurred among German soldiers in Lapland and prisoners in Yugoslavia during World War II (5). About 14,000 cases of war nephritis clinically similar to NE were described among Northern Armies in the American Civil War (6).

In South Korea, 500 to 900 persons are hospitalized annually with this disease and about one third of these are soldiers (7). There were about 114,000 cases of HFRS in China in 1986 with 7% mortality, and several hundred cases of HFRS occurred in other countries of Asia and Europe (8,9).

The causative agent of KHF was first discovered in 1976 from Apodemus mice (10) and isolated from KHF patients in 1978 (11). This agent has been propagated in a human cell culture line (12), and it was named Hantaan virus after the Hantaan river which runs along the 38th Parallel between South and North Korea (13). Antigenic features, genetic properties and EM studies indicate that Hantavirus is a new genus of Bunyaviridae (14-17). A close etiological relationship has been established between KHF and hemorrhagic nephroso-nephritis in the USSR, NE in Scandinavia, EHF in Greece and Eastern Europe, Japan and China (11,18-21).

The working group on HFRS at a WHO meeting in Tokyo, 1982 recommended that all of the above diseases with different names should be referred to as "Haemorrhagic Fever with Renal Syndrome (HFRS)" (22). Recent sero-epidemiologic surveys established that Hantaviruses are widely distributed throughout the world (23-28).

Intraspecific transmission of Hantaan virus in Apodemus mice (29) has also been shown. Infection occurred among cagemates up to 360 days after exposure, while large amounts of virus were excreted in urine and saliva. No evidence for the participation of ectoparasites in virus transmission was found. Infection with Hantaan virus is silent in animals (30), but is associated with diverse clinical symptoms in human (28).

A severe form is common in East Asia, while most European cases are mild (28). The disease is most often sporadic, but under special circumstances epidemics occur. Although predominantly associated with rural areas, HFRS is now being recognized as an urban problem in some countries (28,31,32) and a particular hazard to laboratory staff using rodents for biomedical research (28,33,34). From 1975 to 1986, about 160 cases of HFRS of which one was fatal, occurred in 34 animal rooms of research Institutes in Korea, Japan and Europe among personnel of the animal rooms as a result of exposure to infected rat colonies. Seventy-one % of Korea rats and 40% of the Japanese rats had antibodies to Hantaan virus. Commercial rabbits bought from breeding firms in Korea and Japan were seropositive to Hantaan virus and serum antibodies were found in 3.5% of 792 New Zealand rabbits (35). We have registered a Hantaan related virus isolated from an urban rat caught in Seoul, 1980 as Seoul virus in 1985 (13). Several strains of Seoul virus were isolated from urban rats caught in Korea and Japan and many strains of Hantaan and a Seoul virus were isolated from blood of HFRS patients in Vero E6 cell cultures (7).

Recently, there have been several outbreaks of acute hemorrhagic diseases among soldiers and farmers before and during the epidemic season of HFRS in Korea and it was confirmed that leptospirosis, scrub typhus and other rickettsiosis are the hemorrhagic diseases existing in Korea (7,36).

There are still many problems to be answered in research work of HFRS and some important issues are: a) global survey of Hantavirus infection and HFRS b) serologic relation of hantaviruses isolated from the different parts of the world c) development of a simple serologic diagnostic test d) pathogenesis of hemorrhages and nephritis and e) development of an effective vaccine.

This report describes 1) seroepidemiologic survey of HFRS and other hemorrhagic diseases in Korea 2) development of a simple and rapid serologic diagnostic test for HFRS using high density particles and 3) geographical distribution of HFRS and hantaviruses.

MATERIALS AND METHODS

Collaborations

Since 1980, the WHO Centre for Hemorrhagic Fever with Renal Syndrome has been collaborating with laboratories throughout the world in order to do a seroepidemiologic survey of hantavirus infections in humans and animals. Investigators at the Center collected and received hantaviruses from patients and from rodents.

The Centre supplied hantaviruses isolated from KHF patients and rodents collected in Korea, Japan, Egypt, Hong Kong, Singapore and Sri Lanka to Drs. J.M. Dalrymple, G.R. French, C D. Gajdusek in U.S.A., Dr. C. Y. Kang in Canada, Drs. T. Umenai, J. Kawamata, T. Yamanishi, T. Kitamura, T. Tamura, K. Yamanouchi, A. Tanaka, T. Tomiyama in Japan, Dr. G. van der Groen in Belgium, Drs. G. Song, L. Fang, T. Hung in China, Dr. J. Pilaski in Federal Republic of Germany, Dr. C.Y. Cheng in Singapore, Dr. T. Vitarana in Sri Lanka, Dr. R.J. Kim in N. Korea, Dr. E.A. Tkachenko in USSR, Dr. T. Chang in Taiwan, Dr. M. Weisenbacher in Argentina. We received some strains of hantaviruses isolated from rodents collected in the U.S.A., Japan, Thailand, Brazil, USSR, Finland and Federal Republic of Germany from Drs. J.M. Dalrymple, J.M. LeDuc, P.W. Lee, L.J. Baek, T. Kitamura, T. Yamanishi, G. van der Groen, E.A. Tkachenko, G. Song, J. Pilaski. We received human and animal sera from Drs. N.Y. Agustino, S.B. Villarubio, J. Cross in Philippines, Drs. T.W. Wang, C.Y. Cheong in Singapore, Drs. S. Ambu, T.W. Lim, S.K. Lam in Malaysia, Drs. W.K. Chang, K.F. Shortridge in Hong Kong, Drs. T. Tamura, J. Kawamata, T. Yamanouchi, H. Takada, T. Tanaka, T. Ishida, T. Takagi and K. Miyamoto in Japan, Dr. J.U. Mataika in Fiji, Dr. T. Chang in Taiwan, Dr. H. Hoogstraal in Egypt, Drs. A. Diwan, B.H. Hyun, Y.M. Kim in U.S.A., Dr. M. Weissenbacher in Argentina, Drs. I.H. Chu, M. Kalunda in Uganda, Drs. G. Song, L. Jiang in China, P. Thongcharoen in Thailand, K. Benerjee in India, Dr. M. Nuti in Italy, Drs. J. Laehdevirta, A. Vaheri in Finland, Dr. F.P. Pinheiro in Brazil, Drs. J. Pilaski, H. Will, L. Zoller in Federal Republic of Germany, Dr. D. Chastal in France, Dr. C.Y. Kang in Canada, to investigate the distribution of antibodies to hantaviruses.

In addition, we received monoclonal antibodies prepared against Hantaan virus from Drs. J.M. Dalrymple, J. McCormick, and K. Yamanishi.

Collection of field and urban rodents

Field and house rodents were captured by means of baited live traps and normal Apodemus mice were captured on Jeju island as described (11,14). Seronegative Apodemus mice and Wistar rats were used as sensitive detectors for Hantavirus isolation.

Processing rodents

Living rodents were identified and bled by cardiac puncture under chloroform anesthesia. Serum was separated for antibody titration. Necropsy tissue include lungs, spleen, liver, and kidneys. A portion of each organ was examined immediately by IFA for Hantavirus antigen and the remaining portion were frozen at -70°C until processing for virus isolation.

Specimens from patients

Sera collected from suspected HFRS patients in Korea were used for serodiagnosis. Larger amounts of hyper-immune convalescent serum was collected from HFRS patients for experimental use.

Viruses

The 42 hantaviruses studied were from HFRS patients and rodents collected in different parts of the world (Table 17). These were used after propagation in Vero E6 cell cultures. ID_{50} of the viruses in Vero E6 cells was $10^{5.0} - 10^{6.0}$/ml.

Serum samples

Sera from normal healthy persons including laboratory workers, HFRS patients, rodents and other mammals in different parts of the world were tested for antibodies against hantaviruses.

Antisera against hantaviruses

Two 4 to 5 week old S.D. rats were immunized with each virus by giving them a single intramuscular inoculation of 0.5 ml supernatant fluid from infected Vero E6 cell cultures. Whole blood was collected by cardiac puncture 28 days after inoculation of the virus and serum was separated from the clot, stored at -60° C, and then tested for antibody against the homologous virus and other hantavirus isolates. Serum samples from convalescent-phase HFRS patients in Korea and Finland, and monoclonal antibodies to Hantaan virus were used for comparative serologic studies of hantaviruses.

Antibodies

Neutralizing (N) antibodies to hantaviruses were measured by plaque reduction neutralization test (PRNT) and monoclonal antibodies to hantaviruses were titrated by indirect immunofluorescent antibody technique (IFAT), as described previously (37).

ELISA test

This test for demonstration of IgG and IgM antibodies against Hantaan virus antigen was developed recently, however, it can not differentiate Hantaan virus infection from Seoul virus infection because of cross reaction between them and the method is as described (37).

Antibody test against rickettsiosis

R. tsutsugamushi, R. typhi and R. sibirica strains were obtained from US Army Medical Research Unit in Malaysia. Antigens were prepared in Yolk sac and micro-immunofluorescent antibody technique were used for antibody titration (36)

Carrier particles

High density composite particles (38) (HDP, Tokuyama Soda Co., Tokyo, Japan) were used as carrier. They have a silica core surrounded by a red dye layer and second

silica layer covered the dyed layer. Furthermore, the particle surface is covered with functional groups designed to adsorb antigen. The density of the particles is 2.0 and their diameter is 1.8 μm.

Hantaan-HDPA antigen

Hantaan virus, ROK84-105 strain (39), isolated directly in Vero E8 cell cultures in 1984 from blood of a HFRS patient was used in the HDPA experiments. The virus was passaged 7 times in suckling rat brains to increase titers and virus yield. The LD_{50} of strain ROK84-105 in suckling rats by intracerebral inoculation was $10^{8.5}$/ml. Supernatant fluid of 5% suckling rat brain suspension in phosphate buffered saline (PBS), pH 7.2 was inactivated with 0.05% formalin at 4 C for 15 days. Purification of the inactivated virus suspension for use as antigen in HDPA was done according to the modified method used for preparation of Japanese encephalitis mouse brain vaccine (39). Protein content of the purified antigen preparation was 43 μg/ml and antigen concentration of the preparation was 10,240 units/ml by ELISA test.

Preparation of Hantaan antigen coated HDP

For preparation of Hantaan virus antigen coated HDP (Hanta-HDP), an equal volume of eight ELISA units/ml of Hanta-HDP antigen in PBS was added to the 0.5% HDP suspension in PBS in 1/60 mole, and incubated two hours at 20 C shaking each ten minutes. Then the HDP were washed with PBS twice, suspended in 0.1 the original volume of PBS containing 0.01% bovine serum albumin, 1% dextran, 1% sodium glutamate and 0.5% glycine as a stabilizer, and then lyophilized. Uninfected rat brains were also treated in same manner as antigen control.

Sera from HFRS patients for Hantaan HDPA

Seventeen sera from HFRS patients from Korea (K1-K8), Japan(J1-J4), Finland (F1-F4) and 2 antibody positive sera from healthy persons in the U.S.A., and 4 sera (K9-K12) from people who were inoculated with inactivated Hantaan virus vaccine in Korea were used for antibody determinations. Antibody negative serum from a healthy person in the U.S.A. was also used as negative control serum. All serum samples from HFRS patients and from vaccinees from Korea had been shown in other tests to contain antibodies against Hantaan virus. Likewise, serum samples from HFRS patients in Japan and from healthy people in the U.S.A. were known to contain antibodies to Seoul virus and serum samples from Nephropathia epidemica patients in Finland contained antibodies to Puumala virus.

Procedure of Hantavirus HDPA test

A microtiter techniques was used for HDPA tests for antibodies against Hantaan virus. The virus antigen and normal antigen coated HDP were suspended to a concentration of 0.5% with buffer.

RESULTS

A. Seroepidemiological survey of HFRS and other hemorrhagic diseases among suspect HFRS patients in Korea in 1989.

1. Epidemiologic features of HFRS

There were 430 hospitalized cases of HFRS confirmed serologically at our Institute in 1989 and 3 of them were US Army soldiers as shown in Table 1. Total no. of serum from suspected HFRS in 1989 examined against Hantaan virus was 1,886 and only 22% of them were HFRS patients as shown in Table 2. The ratios of serologically confirmed HFRS patients among clinically suspected HFRS patients by civilian doctors and ROK Army and US Army doctors are about 19%, 82% and 15%, respectively as shown in Table 3. It is noteworthy that ratio of confirmed cases to suspected cases in 1980s is lower than that of 1970s because clinicians have sent us sera from only severe cases of suspect HFRS in 1970s while doctors are sending us more sera from mild suspect HFRS patients and other hemorrhagic fever patients in these years.
Clinicians have made better clinical diagnosis of HFRS during the epidemic season, October-December, than non-epidemic season of HFRS as shown in Table 3.
Patients occur throughout the year and there are two peaks, a small peak in May-July, and a large peak in October-December. One of the new epidemiologic features of HFRS in Korea is the increasing number of HFRS patients in urban areas of Seoul city and one case of HFRS was confirm in Jeju island for the first time as shown in Table 4. There were 80 cases of HFRS in Seoul in 1989. These patients were only hospitalized severe cases, and usually moderate and mild cases are not included because Seoul virus infection in urban areas is mild and usually diagnosed clinically as influenza or unknown fever.
HFRS cases occurred in all districts of Seoul as shown in Table 5. Male patients are the dominant group of HFRS as shown in Table 6 although 104 male soldier patients were not included in this table. Table 4 shows the distribution of HFRS among civilians and about 80% of the patients were in Seoul, Kyunggido, Chungcheongnamdo, and Kangwondo, northern parts of South Korea. Almost all HFRS patients among Korean soldiers occurred in Kyunggido and Kangwondo where main forces of Korean Army is stationed as shown in Table 7. All 3 HFRS patients among U.S. Army soldiers occurred in Kyunggido where the 2nd Division of U.S. Army is stationed.

Table 1.
Hospitalized cases of Hemorrhagic fever with renal syndrome patients in the Republic of Korea.

Year	Korean civilian	Korean soldiers	US soldiers	Total
1951	...	26	827	853
1952	...	18	833	851
1953	...	...	455	455
1954	19	...	307	326
1955	...	...	20	20
1956	...	26	28	54
1957	...	21	13	34
1958	...	20	15	35
1959	...	47	79	126
1960	...	185	10	195
1961	...	341	27	368
1962	...	311	29	340
1963	...	257	11	268
1964	18	205	22	245
1965	2	110	99	211
1966	11	82	36	129
1967	13	86	31	130
1968	26	102	28	156
1969	48	134	9	191
1970	131	221	13	365
1971	391	358	2	751
1972	186	203	0	389
1973	241	237	0	478
1974	176	251	0	427
1975	466	370	1	837
1976	585	304	4	893
1977	288	241	7	536
1978	207	168	10	385
1979	241	122	1	364
1980	185	72	1	258
1981	377	164	2	543
1982	378	123	3	504
1983	402	98	3	503
1984	568	156	6	730
1985	531	159	7	697
1986	530	166	14	710
1987	533	163	5	701
1988	264	97	6	367
1989	320	107	3	430
Total	7,137	5,751	2,967	15,855

Numbers of patients since 1978 are serologically confirmed cases at The Institute for Viral Diseases, Korea University.

Table 2.
Total number of Hemorrhagic fever with renal syndrome (HFRS), murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis patients diagnosed serologically among suspect hemorrhagic fever patients in Korea, 1989.

Disease	No. of patients No. of serum tested
HFRS	430/1886 (22 %)
Murine typhus	359/1886 (19 %)
Scrub typhus	358/1886 (19 %)
SFG rickettsiosis	83/1886 (4 %)
Leptospirosis	28/1886 (1 %)
Unknown	626/1886 (34 %)

Table 3.
Number of serologically confirmed cases of Hemorrhagic fever with renal syndrome patients at The Institute for Viral Diseasaes, Korea University in Korea in 1989.

Month	No. of antibody positive sera against Hantaan virus / No. of tested sera from suspected patients			
	Civilian	ROK Army	Us Army	Total
1	3/48	1/18	0/0	4/66
2	6/35	2/8	0/2	8/45
3	4/41	2/6	1/4	7/51
4	5/42	2/2	1/2	8/46
5	10/73	1/1	0/1	11/75
6	4/70	3/8	0/3	7/81
7	7/62	3/7	0/1	10/70
8	2/60	1/6	0/2	3/68
9	10/90	1/2	1/4	12/96
10	29/238	12/21	0/2	41/261
11	150/683	43/54	0/0	193/737
12	90/247	36/41	0/0	126/288
Total (%)	320/1,689 (19 %)	107/174 (62 %)	3/21 (15 %)	430/1,884 (23 %)

Table 4.
Geographical distribution of confirmed cases of Hemorrhagic fever with renal syndrome patients among civilian in Korea in 1989 at The Institute for Viral Diseases, Korea University.

Name of province	Month												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Seoul city	0	2	2	3	5	1	6	1	6	12	30	12	80
Kyounggi-do	1	3	2	2	3	2	1	1	2	7	60	37	121
Kangwon-do	0	0	0	0	2	0	0	0	1	3	15	9	30
Chungcheongbuk-do	1	0	0	0	0	1	0	0	1	4	0	2	9
Chungcheongnam-do	0	0	0	0	0	0	0	0	0	0	27	17	44
Kyungsangbuk-do	0	0	0	0	0	0	0	0	0	0	4	3	7
Kyungsangnam-do	1	0	0	0	0	0	0	0	0	0	2	2	5
Jeollabuk-do	0	0	0	0	0	0	0	0	0	1	0	1	2
Jeollanam-do	0	1	0	0	0	0	0	0	0	1	12	7	21
Jeju-do	0	0	0	0	0	0	0	0	0	1	0	0	1
Total	3	6	4	5	10	4	7	2	10	29	150	90	320

Table 5.
Occurrence of HFRS patients in districts of Seoul city in 1989.

Name of district	No. of patients	Name of district	No. of patients
Yongsan-ku	3	Joong-Ku	4
Seongbuk-ku	2	Jungryang-ku	1
Seongdong-ku	7	Kwanak-ku	4
Yeongdeungpo-ku	4	Songpa-ku	3
Dobong-ku	11	Eunpyung-ku	3
Dongdaemun-ku	6	Kangseo-ku	4
Kuro-ku	4	Mapo-ku	3
Chongro-ku	2	Seocho-ku	4
Dongzak-ku	3	Seodaemun-ku	1
Kangdong-ku	8	Kangnam-ku	0
Nowon-Ku	1	Yangcheon-ku	2
		Total	80

Table 6.
Age and sex distribution of HFRS, murine typhus, scrub typhus, spotted fever and leptospirosis among civilian patients in 1989 in Korea.

Age	HFRS			murine typhus			scrub typhus			spotted fever			leptospirosis		
	M	F	total	M	F	total	M	F	total	M	F	total	M	F	total
0-10	3	0	3	3	1	4	3	1	4	2	2	4	0	1	1
11-20	15	5	20	5	7	12	3	3	6	4	1	5	2	0	2
21-30	47	12	59	27	12	39	11	5	16	8	8	16	5	2	7
31-40	48	17	65	29	17	46	13	17	30	5	8	13	2	1	3
41-50	44	9	53	34	28	62	13	20	33	6	5	11	1	1	2
51-60	33	22	55	48	19	67	23	63	86	6	5	11	6	0	6
61-70	13	14	27	33	33	66	27	49	76	2	3	5	4	0	4
71-80	4	2	6	7	12	19	14	20	34	2	1	3	1	0	1
unknown	23	9	32	15	14	29	37	30	67	8	3	11	1	0	1
Total	230 (72%)	90 (28%)	320 (100%)	201 (59%)	143 (41%)	344 (100%)	144 (41%)	208 (59%)	352 (100%)	43 (55%)	36 (45%)	79 (100%)	22 (82%)	5 (18%)	27 (100%)

M : Male F: Female

Table 7.
Geographical occurrence of HFRS patients among ROKA soldiers in 1989 in S. Korea.

Name of area	No. of patient	Name of area	No. of patient
Seoul city	3	Kangwondo	
Kyunggido		Whacheon	12
Paju	19	Chulwon	11
Yeoncheon	17	Chunseong	5
Pocheon	6	Yangku	4
Koyang	4	Inje	3
Yangju	3	Koseong	2
Yangpyung	2	Hongcheon	1
Kimpo	2	Wontong	1
Wondang	1	Chungcheongdo	
Eujeongbu	1	Nonsan	1
Munsan	1	Youngdong	1
Suwon	1	Hongseong	1
Songtan	1	Kyungsangdo	
Kangwha	1	Yeacheon	1
Kapyung	1		
Jeokseong	1		
		Total: 107 patients	

2. Outbreaks of acute febrile hemorrhagic diseases during the epidemic season of HFRS in 1989.

As shown in Tables 2 and 8, the no. of confirmed cases of civilian scrub typhus was 352 among 1,686 suspect HFRS. These sera from the hospitalized patients were sent to our laboratory from hospitals in and nearby Seoul city for serologic diagnosis of HFRS. The no. of scrub typhus patients among ROK Army and U.S. Army is 6 and none, respectively. Most of rickettsiosis patients occurred in October and November, about a month before the large epidemic season of HFRS as shown in Table 9. Geographical distribution of scrub typhus patients in South Korea is shown in Table 8 and most of the patients were confirmed in Jeollanam-do, Kyunggi-do, Seoul city, Kangwon-do, and Kyungsangnam-do, and many patients also occurred in other provinces as well. About 60% of scrub typhus patients among civilians were female and about 90% of the patients were in the age group of over 31 as shown in Table 6. A large outbreak of murine typhus was demonstrated in 1989 as shown in Tables 2 and 8. It is noteworthy that murine typhus occurred in every month of the year and most of the patients were distributed in Seoul, Kyunggi-do, Kangwon-do, Chungcheongnam-do, Jeollanam-do and Kyungsangnam-do. Of 344 murine typhus patients, 201 were male and 143 were female as shown in Table 6. A large outbreak of spotted fever group of rickettsiosis was also demonstrated as shown in Tables 2, 6, 8 and 10. An epidemic of spotted fever occurred in summer and fall. Twenty-seven cases of leptospitosis were diagnosed serologically among 1,686 suspect HFRS patients as shown in Tables 2, 6 and 9. Many cases of leptospirosis were found in Seoul and Kyunggi-do, and about 80% of them were male.

In the Korean Army, HFRS is a major military problem and murine typhus and spotted fever are next as shown in Tables 10 and 11. In U.S. Army in Korea, HFRS and murine typhus were confirmed serologically in 1989 as shown in Tables 13 and 14.

B. Hantaan coated high density particle (HDP) agglutination against hantavirus antibodies.

Box titration of antigen coated HDP and antibody in human serum was carried out as shown in Table 15. Positive agglutination patterns using antigen coated HDP were clearly demonstrable against antibodies after 40 minutes incubation at room temperature. The optimum antigen coating concentration was four to eight ELISA units/ml, while negative reactions were found with diluent. Alternatively, non-coated HDP antigen controls were always negative against both

Table 8.
Geographical distribution of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect civilian hemorrhagic fever patients in Korea, 1989.

Name of province	No. of case				
	HFRS	murine typhus	scrub typhus	spotted fever	lepto-spirosis
Seoul city	80	121	48	30	12
Kyunggi-do	121	73	48	18	9
Kangwon-do	30	28	25	8	1
Chungcheongbuk-do	9	16	16	2	0
Chungcheongnam-do	44	21	37	7	2
Kyungsangbuk-do	7	16	12	2	0
Kyungsangnam-do	5	18	38	2	1
Jeollabuk-do	2	7	15	0	1
Jeollanam-do	21	42	110	8	1
Jeju-do	1	2	3	2	0
Total	320	344	352	79	27

Table 9.
Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect civilian hemorrhagic fever patients in Korea, 1989.

Month	HFRS	murine typhus	scrub typhus	spotted fever	lepto-spirosis	unknown
1	3/48	4/48	0/48	0/48	0/48	41/48
2	6/35	2/35	0/35	0/35	0/35	26/35
3	4/41	7/41	1/41	0/41	7/41	22/41
4	5/42	1/42	1/42	0/42	1/42	34/42
5	10/73	6/73	0/73	0/73	1/73	56/73
6	4/70	13/70	2/70	1/70	3/70	47/70
7	7/62	22/62	7/62	15/62	0/62	11/62
8	2/60	39/60	1/60	6/60	2/60	10/60
9	10/90	29/90	2/90	6/90	8/90	35/90
10	29/238	53/238	76/238	4/238	2/238	74/238
11	150/683	123/683	248/683	9/683	0/683	153/683
12	90/247	45/247	14/247	38/247	2/247	58/247
Total	320/1686 (19 %)	344/1686 (21 %)	352/1686 (21 %)	79/1686 (5 %)	27/1686 (2 %)	564/1686 (34 %)

Table 10.
Monthly distribution of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) and leptospirosis among suspect civilian hemorrhagic fever patients by sex in Korea, 1989.

Month	HFRS			murine typhus			scrub typhus			spotted fever			leptospirosis		
	M	F	total	M	F	total	M	F	total	M	F	total	M	F	total
1	3	0	3	0	4	4	0	0	0	0	0	0	0	0	0
2	4	2	6	1	1	2	0	0	0	0	0	0	1	0	1
3	2	2	4	4	3	7	1	0	1	0	0	0	6	1	7
4	3	2	5	0	1	1	1	0	1	0	0	0	0	1	1
5	7	3	10	3	3	6	0	0	0	0	0	0	1	0	1
6	2	2	4	6	7	13	1	1	2	1	0	1	3	0	3
7	5	2	7	17	5	22	4	3	7	11	4	15	0	0	0
8	1	1	2	23	16	39	1	0	1	4	2	6	2	0	2
9	9	1	10	20	9	29	2	0	2	3	3	6	5	3	8
10	19	10	29	34	19	53	27	49	76	1	3	4	2	0	2
11	105	45	150	62	61	123	102	146	248	5	4	9	0	0	0
12	70	20	90	31	14	45	5	9	14	18	20	38	2	0	2
Total	230 (72%)	90 (28%)	320 (100%)	201 (59%)	143 (41%)	344 (100%)	144 (41%)	208 (59%)	352 (100%)	43 (55%)	36 (45%)	79 (100%)	22 (82%)	5 (18%)	27 (100%)

M : Male F: Female

Table 11.
Number of HFRS, scrub typhus, murine typhus and spotted fever diagnosed serologically among suspect HFRS patients clinically in ROK soldiers at The Institute for Viral Diseases, Korea University in Korea, 1989.

Total no. of HFRS / Total no. of serum tested	※ =	107 / 174	(61 %)
Total no. of scrub typhus / Total no. of serum tested	=	6 / 174	(3 %)
Total no. of murine typhus / Total no. of serum tested	=	14 / 174	(8 %)
Total no. of spotted fever / Total no. of serum tested	=	4 / 174	(2 %)
Total no. of leptospirosis / Total no. of serum tested	=	1 / 174	(0.6 %)
Total no. of unknown sera / Total no. of serum tested	=	44 / 174	(25 %)

※ No. of confirmed patient serologically / No. of serum from suspect HFRS patient tested

Table 12.
Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect ROKA soldiers at The Institute for Viral Diseases, Korea Uiversity in Korea, 1989.

Month	HFRS	murine typhus	scrub typhus	spotted fever	lepto-spirosis
1	1/18	0/18	0/18	0/18	0/18
2	2/8	1/8	0/8	0/8	0/8
3	2/6	1/6	1/6	0/6	1/6
4	2/2	0/2	0/2	0/2	0/2
5	1/1	0/1	0/1	0/1	0/1
6	3/8	2/8	0/8	0/8	0/8
7	3/7	1/7	0/7	0/7	0/7
8	1/6	5/6	0/6	0/6	0/6
9	1/2	0/2	0/2	0/2	0/2
10	12/21	3/21	0/21	1/21	0/21
11	43/54	1/54	5/54	0/54	0/54
12	36/41	0/41	0/41	0/41	0/41
Total	107/174 (61 %)	14/174 (8 %)	6/174 (3 %)	4/176 (2 %)	1/174 (0.6 %)

Table 13.
Number of HFRS, scrub typhus, murine typhus and spotted fever diagnosed serologically among suspect HFRS patients clinically in US Army soldiers at The Institute for Viral Diseases, Korea University in Korea, 1989.

Total no. of HFRS / Total no. of serum tested	[V] =	3 / 21	(14 %)
Total no. of scrub typhus / Total no. of serum tested	=	0 / 21	(0 %)
Total no. of murine typhus / Total no. of serum tested	=	1 / 21	(5 %)
Total no. of spotted fever / Total no. of serum tested	=	0 / 21	(0 %)
Total no. of leptospirosis / Total no. of serum tested	=	0 / 21	(0 %)
Total no. of unknown sera / Total no. of serum tested	=	17 / 21	(81 %)

[V] No. of confirmed patient serologically / No. of serum from suspect HFRS patient tested

Table 14.
Monthly incidence of HFRS, murine typhus, scrub typhus, spotted fever group (SFG) rickettsiosis and leptospirosis among suspect US Army soldiers at The Institute for Viral Diseases, Korea University in Korea, 1989.

Month	HFRS	murine typhus	scrub typhus	spotted fever	lepto-spirosis
1	0/0	0/0	0/0	0/0	0/0
2	0/2	0/2	0/2	0/2	0/2
3	1/4	0/4	0/4	0/4	0/4
4	1/2	0/2	0/2	0/2	0/2
5	0/1	0/1	0/1	0/1	0/1
6	0/3	0/3	0/3	0/3	0/3
7	0/1	0/1	0/1	0/1	0/1
8	0/2	0/2	0/2	0/2	0/2
9	1/4	0/4	0/4	0/4	0/4
10	0/2	0/2	0/2	0/2	0/2
11	0/0	0/0	0/0	0/0	0/0
12	0/0	0/0	0/0	0/0	0/0
Total	3/21 (14 %)	0/21 (0 %)	1/21 (5 %)	0/21 (0 %)	0/21 (0 %)

diluent and serum containing antibodies. Comparative antibody titers of sera from HFRS patients and from individuals vaccinated with Hantaan virus, as determined by IFAT, ELISA and HDPA, are shown in Table 16. Hantaan virus antigen-coated HDP reacted with not only antibody to Hantaan virus (sera from Korea) but also with antibodies to Seoul virus (sera from Japan and U.S.A.) and with antibodies to Puumula virus (sera from Finland), as was shown in parallel IFAT and ELISA. It was also found that HDPA titers were about two to ten times higher than IFA titers but that ELISA antibody titers usually were higher than HDPA and IFAT.

C. Global distribution of humans and rodents infected with hantaviruses.

Seroepidemiological surveys show that hantavirus infections are distributed throughout much of the world, as demonstrated by the presence of antibodies against hantaviruses in sera from humans and rodents (28), as shown in Fig. 1.

HFRS patients have been documented clinically and serologically throughout Eurasia and, recently, in Africa. Nine countries in Asia are focally enzootic for hantaviruses: Japan, South Korea, North Korea, China, Mongolia, U.S.S.R., Hong Kong, Malaysia and Sri Lanka; 15 countries in Europe: U.S.S.R., Finland, Sweden, Norway, Denmark, Bulgaria, Hungary, Albania, Federal Republic of Germany, France, Belgium, Netherland, England, Yugoslavia and Greece; and 1 country in Africa: Central African Republic (40).

Severe and moderate clinical forms of HFRS occur in Asia and in Balkan countries. Annually, between 70,000 and 130,000 people are hospitalized with HFRS in China, about 500-800 in South Korea, several hundred in North Korea, and several hundred in the U.S.S.R. Recently, several cases of HFRS were documented in Malaysia and Sri Lanka. Most HFRS patients in Asia live in rural areas but there have been many infections acquired in urban areas of Japan, Korea, China and Hong Kong. In Europe, HFRS is usually a more mild illness, known as Nephropathia Epidemica, in which renal involvement dominates and hemorrhagic features are less prominent; the fatality rate is about 0.2%. However, the severe type occurs in parts of Yugoslavia and Greece.

Infections of laboratory workers with Hantaan and Seoul viruses have been reported from Korea, Japan, China, Belgium, and England (28). Thus, seropositive humans and wild rodents have been determined almost world-wide.

Table 15.
Box titration of the Hantaan virus antigen-coated HDP and antibody positive serum.

Hantaan virus antigen (ELISA U/ml)	Dilution of antibody positive serum[a]						
	100	200	400	1600	3200	6400	Diluent
16	+++	+++	+++	+++	+++	+	+
8	+++	+++	+++	+++	+++	-	-
4	+++	+++	+++	+++	+++	-	-
2	+++	+++	++	-	-	-	-
1	-	-	-	-	-	-	-
0	-	-	-	-	-	-	-

a) Antibody titer of serum from this HFRS patients was 1:512 by IFAT.

Table 16.
Comparative antibody titers of sera from HFRS patients and vaccinees against Hantaan virus by HDPA, IFAT and ELISA.

Origin of country	Code no. of serum	Antibody titer to Hantaan virus by HDPA	IFAT	ELISA
Japan	J-1	4,000	1,048	25,600
	J-2-1	320	256	3,200
	J-2-2	320	256	3,200
	J-3	4,000	512	25,600
	J-4	640	512	6,400
U. S. A.	US-1	1,000	64	800
	US-2	<40	512	<100
Finland	F-1	160	128	6,400
	F-2	40	32	3,200
	F-3	160	1,024	6,400
	F-4	320	1,024	6,400
Korea	K-1	16,000	1,024	25,600
	K-2	8,000	4,096	25,600
	K-3	16,000	2,048	25,600
	K-4	4,000	2,048	25,600
	K-5	1,600	1,024	6,400
	K-6	12,800	4,096	25,600
	K-7	12,800	4,096	6,400
	K-8	6,400	4,096	6,400
	K-9	3,200	256	6,400
	K-10	3,200	256	6,400
	K-11	200	253	800
	K-12	3,200	128	1,600
U.S.A.	Negative control	<40	<16	<100

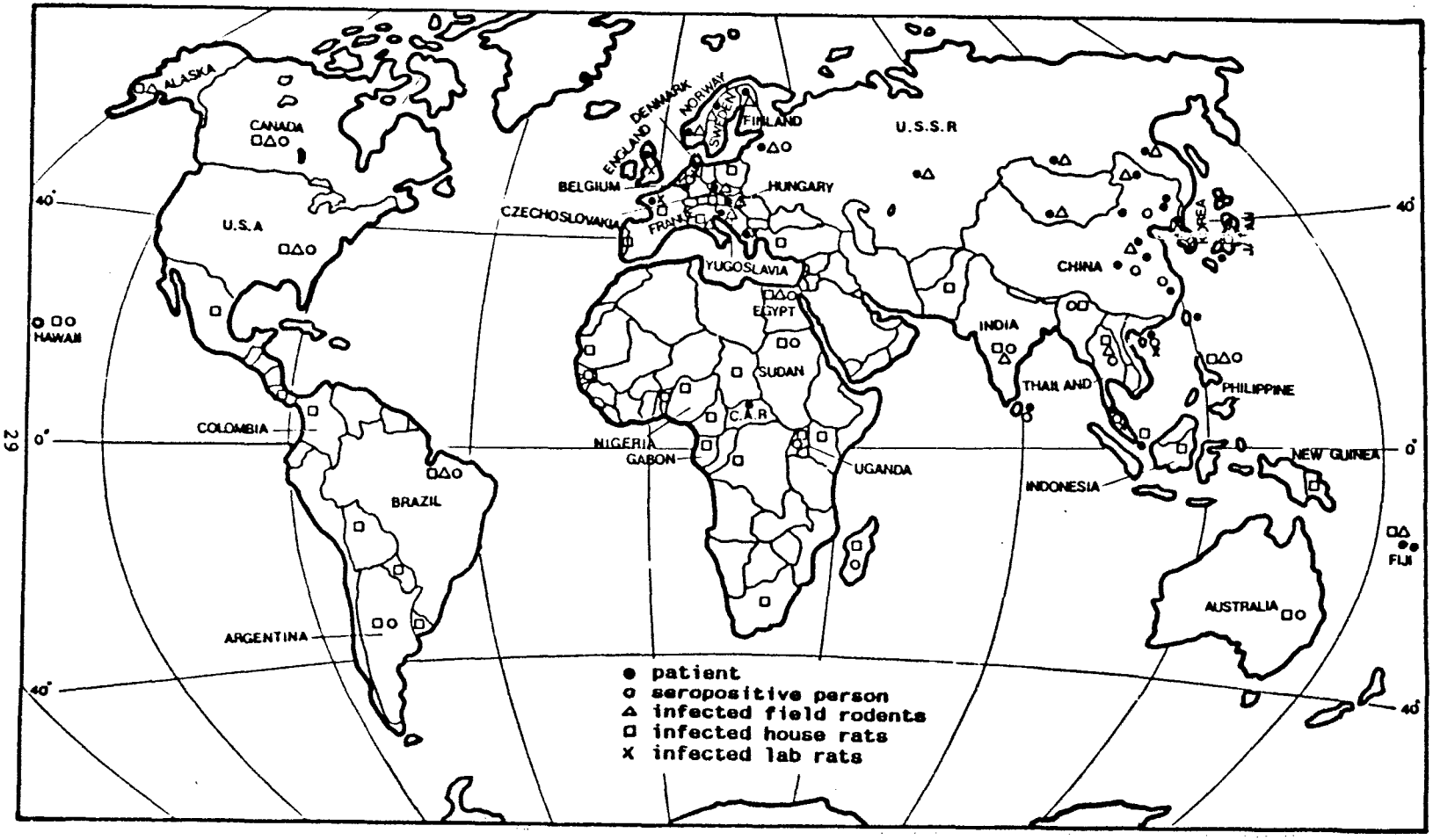

Fig. 1.

Global distribution of humans with HFRS and humans and rodent infected with hantaviruses, based on demonstration of antibodies against hantaviruses.

Field rodents infected with hantaviruses were demonstrated in Asia, Europe, Africa and the Americas, specifically: Korea, China, U.S.S.R., Sweden, Finland, Norway, Yugoslavia, Greece, Egypt, U.S.A., Brazil, and Argentina. Urban rats infected with hantaviruses are in 10 Asian countries: Japan, Korea, China, Hong Kong, Malaysia, Sri Lanka, India, Singapore, Fiji, Philippines; in 3 European countries: Belgium, Federal Republic of Germany, Italy; and in the U.S.a. Laboratory rats infected with hantaviruses have been determined in 11 countries of the world: Japan, Korea, China, U.S.S.R., Belgium, England, Malaysia, Hong Kong, Singapore, Hawaii, Argentina.

D. Global distribution of hantaviruses.

The demonstration of N antibodies against different hantaviruses in sera from humans and rodents from different parts of the world indicate geographical distribution of hantaviruses (Fig. 2).

Hantaan virus is found in Korea, China, Mongolia, and the Far-East of the U.S.S.R., Seoul and related viruses exist essentially world-wide: 13 Asian countries (Japan, Korea, China, Hong Kong, Philippines, Malaysia, Singapore, India, Sri Lanka, Fiji, Thailand, Vietnam, Taiwan), 4 North and Central American countries (Canada, U.S.A., Mixico, Panama), 6 South American countries (Brazil, Bolivia, Colombia, Argentina, Uruguay, Paraguay), and 13 African countries (Egypt, Sudan, Uganda, Kenya, Benin, Cameroun, Mauritania, Senegal, Tchad, Central African Republic, Gabon, Madagascar, Nigeria), 4 countries in Europe (Belgium, Netherlands, Federal Republic of Germany, Italy).

Puumala virus is in Europe (U.S.S.R., Scandinavian countries, Finland, Belgium, Federal Republic of Germany, France and England). Prospect Hill and Leaky viruses have been found only in the U.S.A.

Maagi virus, first isolated from Apodemus agrarius collected in Maagi, Kyunggi-do, Korea, 1980 (H.W. Lee, unpubl. data) is found in Korea and probably in Yugoslavia ard Greece, based on demonstration of specific antibodies against the virus in sera from HFRS patients.

E. Serologic relationships between hantaviruses by neutralization tests and monoclonal antibody assays.

Neutralization tests were used to serologically classify 42 strains of hantaviruses isolated from HFRS patients and from rodents in different parts of the world. N antibody titers of rat antiserum to each strain of hantaviruses were measured against 6 ostensibly different serotypes of hantaviruses (Table 17). There were 6 strains of

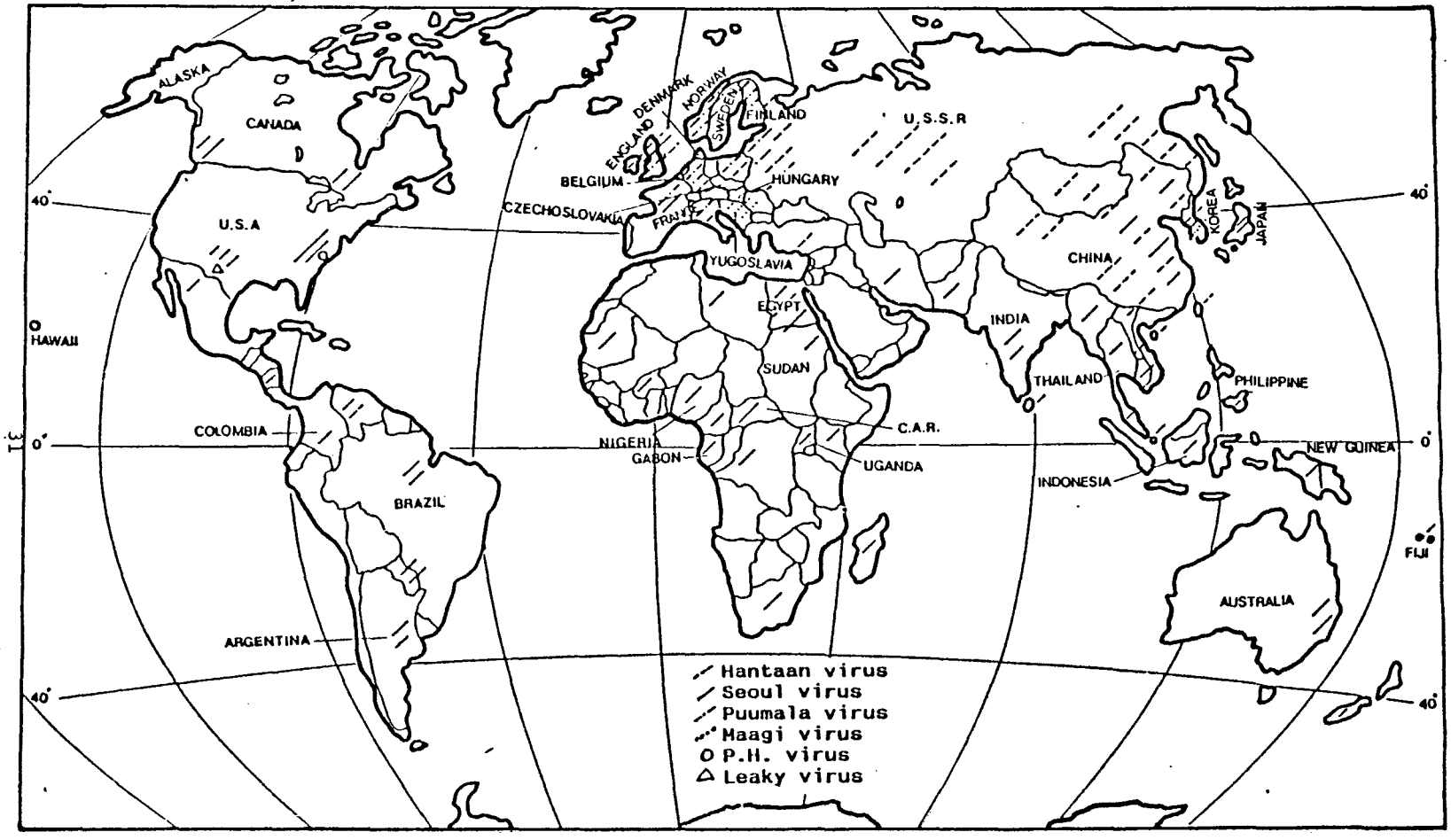

Fig. 2.
Global distribution of hantaviruses based on demonstration of antibodies against the viruses in humans and rodents.

Hantaan virus and 1 of Seoul virus from 7 HFRS patients. Ten strains of Hantaan virus and one strain of Maagi virus were identified among 11 isolates from Apodemus mice.

Eight strains of Seoul virus and 6 strains of Seoul-like virus (equal N antibody titers against Hantaan and Seoul viruses) were identified from 14 isolates from house rats. Of three isolates from laboratory animals, 1 from a hamster and 1 from a bandicoot (Bandicota indica) were Seoul virus, the third isolate was Hantaan virus. One Puumala and 1 Hantaan virus were from Clethrionomys mice. One isolate from Microtus pennsylvanicus was Prospect Hill virus and one isolate from Mus musculus was Leaky virus.

By neutralization, there are 6 distinct serotypes (Hantaan, Seoul, Puumala, Prospect Hill, Maagi, and Leaky) and 1 undistinguishable type isolate (related closely to both Hantaan and Seoul viruses) among 42 hantavirus isolates from humans and animals. However, antisera made with 5 isolates from rats (Egypt R.12915, Tchoupitoulas, JTRN/82/17, TR-352 and I/RN/82-3) showed equal antibody titers to Hantaan and Seoul viruses, these isolates require further study.

Results of antigenic comparison of 38 hantavirus isolates from HFRS patients and from animals in different parts of the world by IFA, using 8 monoclonal antibodies and 3 sera from HFRS patients are shown in Table 18. Ten Hantaan virus strains and Maagi virus were clearly distinguishable from each other and from other hantaviruses using monoclonal antibody BB01-BB08. Twenty-three strains of Seoul virus could be separated into two closely related clusters using monoclonal antibodies HC02-BE08 and HC02-BD05. Three (JTRN82/17, TR-352, I/RN/82/3) of 4 strains of Seoul virus that did not react with monoclonal antibodies HC02-BE08 and HC02-BD05 had shown equal N antibody titers to Hantaan and Seoul viruses by N (Table 17). However, Hubei/1 strain, 1 of 4 that did not react with monoclonal antibodies HC02-BZ08 and HC02-BD05, was essentially identical to Seoul virus by N (Table 17). Maagi virus can be distinguished from Hantaan virus using monoclonal antibodies HC02-BE08, HC02-BD05 and HC02-BD05, as shown in Table 18. Puumala, Prospect Hill, and Leaky viruses were not reactive with the monoclonal antibodies but could be differentiated from other hantaviruses by using convalescent sera from KHF and NE patients (Table 18).

Table 17.
Serologic relation of hantaviruses by PRNT isolated from HFRS patients and rodents in the world.

Rat antiserum to virus	PRN antibody titer against hantavirus					
	Hantaan virus	Seoul virus	Puumala virus	Prospect Hill virus	Maagi virus	Leaky virus
Human isolates:						
ROK 79/89	1,280	20	<20	20	1,280	<20
ROK 84/105	1,280	20	<20	20	320	n.d.
LEE#188604	320	10	<20	<20	320	n.d.
US 84/2	640	20	<20	<20	320	n.d.
Chen	320	20	<20	20	640	n.d.
ROK 79/90	320	20	<20	20	640	n.d.
Hubei/1	80	5,120	<20	<20	320	n.d.
Apodemus isolates:						
76/118	5,120	80	<20	20	5,120	<20
78/197	1,280	20	<20	<20	1,280	n.d.
83/18	1,280	20	<20	<20	1,280	n.d.
83/138	5,120	80	<20	<20	1,280	n.d.
Yugo/2508/84	5,120	80	<20	<20	5,120	n.d.
A9	1,280	20	<20	<20	640	n.d.
Jinhae 87/494	1,280	80	<20	<20	1,280	n.d.
Jinhae 87/502	5,120	80	<20	<20	5,120	n.d.
83/14	320	20	<20	<20	320	n.d.
Jinhae 87/526	640	80	<20	<20	1,280	n.d.
Maagi	320	20	<20	<20	5,120	n.d.

Table 17. (continued)

House rat isolates:						
80/39(#211808)	20	1,280	<20	<20	320	<20
I/RN/82/216	320	20,480	<20	<20	320	n.d.
JTRN/82/11	320	20,480	<20	<20	320	n.d.
Hong Kong R/14	320	1,280	20	20	n.d.	n.d.
Thailand #605	20	1,280	<20	20	80	n.d.
Brazil 2/4	80	5,120	<20	<20	80	n.d.
Singapore R/36	80	1,280	<20	<20	n.d.	n.d.
R22	80	5,120	20	<20	n.d.	n.d.
Girard Point	320	640	<20	<20	320	n.d.
Egypt R/12915	320	320	<20	20	n.d.	n.d.
Tchoupitoulas	320	320	<20	20	80	n.d.
JTRN/82/17	320	320	<20	<20	n.d.	n.d.
TR/352	80	80	<20	<20	n.d.	n.d.
I/RN/82/3	320	320	<20	<20	n.d.	n.d.
Lab. rat isolates:						
KSNUSD 84/30	1,280	160	<20	<20	640	n.d.
KSNUSD 84/34	80	1,280	<20	<20	n.d.	<20
B/1	80	5,120	20	<20	<20	n.d.
SR/11 #191811	80	320	20	<20	n.d.	n.d.
Hamster isolate:						
SNUS/Hamster 85/4	80	5,120	<20	<20	20	n.d.
Bandicota isolate:						
Thailand #749	80	1,280	<20	<20	320	n.d.
Clethrionomys isolates:						
Hållnäs B	<20	80	320	80	80	<20
USSR/CLS1/452	320	20	20	20	n.d.*	n.d.
Microtus isolate:						
Prospect Hill	20	20	320	5,120	n.d.	<20
M. musculus isolate:						
Leaky	<20	<20	<20	<20	n.d.	320

* n.d. : not done

Table 18.
Comparative IFA titers of *monoclonal* antibodies against different strains of Hantavirus isolated from HFRS patients and rodents in the world.

Serotype by PRNT	No. Virus strain	Monoclonal antibodies from Hantaan virus								HFRS patient's	
		BB01-BB08	HC02-BE08	HC02-BD05	FD03-AA11	FD03-AF03	Mc-Ab 33-B	Mc-Ab 40-A	Mc-Ab 80-A	KHF-85-26	NE/Fin 85-797
Hantaan virus	1. KHF 83/61	8,192	256	2,048	256	16	64	32	8,192	4,096	1,024
	2. Lee #188604	8,192	1,024	1,024	128	128	-	-	8,192	2,048	2,048
	3. ROK 84/105	65,536	256	1,024	64	-	256	256	1,024	8,192	2,048
	4. US 84/2	16,384	1,024	1,024	64	-	256	-	1,024	2,048	2,048
	5. 76/118 #050323	8,192	256	1,024	128	128	64	32	4,096	2,048	2.048
	6. USSR/Cl S1/452	65,536	2,048	512	64	32	256	256	1,024	8,192	2,048
	7. KSNUSD 84/30	65,536	4,096	4,096	1,024	64	256	256	2,048	8,192	2,048
	8. 79/89	8,192	64	256	128	-	64	-	8,192	8,192	1,024
	9. 83/14	4,096	64	64	128	-	32	-	8,192	4,096	4,096
	10. 83/183	4,096	64	-	128	-	64	32	8,192	8,192	1,024
Maagi virus	1. Maagi	16,384	-	-	-	-	256	-	1,024	8,192	2,048

Table 18. (continued)

	1. Thailand #605	–	4,096	4,096	–	–	–	128	4,096	4,096	2,048
	2. Thailand #749	–	4,096	4,096	1,024	1,024	–	128	4,096	8,192	2,048
	3. Brazil 2/4	–	4,096	4,096	1,024	1,024	–	256	4,096	4,096	2,048
	4. SNU/Hamster 85/4	64	1,024	1,024	–	64	–	64	2,048	2,048	512
	5. KHF 83/109	–	512	2,048	256	–	–	–	8,192	4,096	4,096
	6. 80/39 #211808	–	1,024	1,024	32	–	–	–	8,192	2,048	2,048
	7. SR/11 #191811	–	1,024	1,024	128	32	–	–	4,096	4,096	4,096
	8. B/1	–	1,024	1,024	128	32	–	–	8,192	2,048	2,048
	9. Girard Point	–	1,024	1,024	128	32	–	–	8,192	4,096	1,024
	10. Tchoupitoulas	–	1,024	1,024	32	32	–	–	8,192	2,048	2,048
Seoul	11. Singapore R/36	64	4,096	4,096	64	64	64	–	4,096	8,192	2,048
virus	12. Hong Kong R/14	–	65,536	1,024	4,096	1,024	–	–	4,096	8,192	4,096
	13. Hong Kong R/35	–	65,536	512	4,096	1,024	–	–	4,096	16,384	4,096
	14. Hong Kong R/40	–	65,536	1,024	4,096	4,096	–	–	4,096	16,384	4,096
	15. Hong Kong R/90	–	65,536	1,024	4,096	1,024	–	–	8,192	16,384	4,096
	16. Egypt R/12915	32	1,024	256	64	64	–	–	4,096	8,192	2,048
	17. Egypt R/13120	–	4,096	4,096	64	64	–	–	4,096	2,048	1,024
	18. SNUSD 84/34	–	256	256	–	64	64	–	4,096	2,048	1,024
	19. Sri Lanka	16	4,096	256	16	16	–	–	4,096	4,096	1,024
	20. Hubei/1	–	–	–	128	128	–	–	8,192	4,096	2,048
	21. JTRN/82/17	–	–	–	256	–	–	–	8,192	1,024	1,024
	22. TR/352	–	–	–	128	32	–	–	8,192	4,096	2,048
	23. I/RN/82/3	–	–	–	–	–	–	–	8,192	1,024	1,024
Puumala virus	1. Hallnas B	64	–	–	–	–	–	–	256	128	16,384
	2. USSR C1/18-20	–	–	–	–	–	–	–	64	64	4,096
Prospect Hill virus	1. Prospect Hill	–	–	–	–	–	–	–	256	256	2,048
Leaky virus	1. Leaky	16	16	n.d.	16	16	n.d.	n.d.	n.d.	256	1,024

DISCUSSION

It has been known that HFRS occurs throughout Korea. The 430 cases of HFRS represent only serologically confirmed hospitalized patients at our Institute in 1989. Sera from suspect HFRS patients came from limited hospitals in and nearby cities of Seoul, therefore, the real total no. of HFRS in entire South Korea should be at least three times more than no. of patients in table 1 because I assume that we might have examined about one third of HFRS cases according to the distribution of population. It could be estimated that there are at least 2,000 cases of HFRS patient in S. Korea every year if serologic diagnostic capabilities are available at the endemic areas of S. Korea. About 90% of total patients were distributed in 4 Provinces located in northern parts of S. Korea as shown in table 4 but it might not mean that the Provinces are more heavily infected foci of HFRS than other Provinces since other Provinces are far from Seoul and it is very difficult to send sera from the suspect patients at acute stage of illness to our Institute for serologic diagnosis of the disease. Distribution of HFRS patient in Seoul city is in all districts and every district had several cases of HFRS every year. It remains to be studied the risk factors and virulence of Seoul virus strains where about 10 million people are living and more than 10% of urban rats population is infected with Seoul virus (32). There is a large epidemic peak of HFRS in late fall and a minor peak in May-July every year and there were 107 cases among Korean soldiers stationed near DMZ between South and North Korea. There were only 3 HFRS out of 21 suspect HFRS patients among about 40,000 U.S. soldiers stationed in Korea. Recently, it has been known that the clinical complex of acute hemorrhagic diseases in summer and fall in Korea since 1982, includes HFRS, leptospirosis and rickettsioses (37). It is surprising to learn that there were 359 cases of murine typhus, 358 cases of scrub typhus and 83 spotted fever patients as shown in table 2. We have reported the evidance for the existence of murine typhus and spotted fever group rickettsiosis in Korea in the 1989 Annual Summary Report.

However, still about 33% of total suspect HFRS patients was not diagnosed serologically and remains to be answered and we are trying to find the etiologic agents of the unknown fevers. We have the serologic evidence of existence of Colorado tick fever like illness in Korea. Collaborative study to search the causative agents of unknown fevers among hemorrhagic diseases between USAMRU/ Korea, USAMRIID, CDC in Colorado and our Institute is in progress.

It is also planned to isolate local strains of R. typhi and R. siberica from wild rodents and their ectoparasites in the near future.

It has been recognized that HDP sensitizes more proteinous and lipoid antigen on their surface, which results in higher sensitivity to antibody than similarly used erythrocytes of polystyrene latex particles (38). As for hantavirus HDPA, we found that this test provides higher sensitivity than IFAT, which may depend on this binding property of HDP.

The highly purified Hantaan virus antigen as here applied had cross reaction with only antisera to Seoul and Puumala viruses that belong to genus hantavirus by Hantavirus HDPA test but neither cross reaction with other etiologic agents of viral haemorrhagic fevers, such as dengue, Rift Valley fever, Crimean-Congo haemorrhagic fever, Junin and Machupo haemorrhagic fever and Ebola, nor non-specific aglutination with sera from bacterial haemorrhagic diseases, such as leptospirosis and rickettsiosis were observed.

Accordingly, as antigen-coated HDP were lyophilized, this reaction is easily used for measurement of hantavirus antibody, without any technical complexity, within 1 hr. The available serologic diagnostic tests for HFRS are IFAT, ELISA, plaque-reduction neutralization test, hemagglutination inhibition test and an immune adherence hemagglutination test (7), but these tests are complicated and time-consuming compared with hantavirus HDPA test. In our studies, only IgG antibodies in sera from FHRS patients and vaccinees were compared by the tests because hantavirus HDPA test can not differentiate IgG and IgM antibodies.

It is expected that this test will be applied for clinical and epidemiological use, especially for rapid serodiagnosis of hantavirus infections among suspect HFRS patients at hospitals in the areas endemic for HFRS.

It has only been within the past decade that concrete progress has been made in the knowledge of the etiology and epidemiology of HFRS. Isolation of Hantaan virus, the prototype virus of HFRS, and development of an IFAT in 1976 for serologic diagnosis of the disease has led to the recognition that hantaviruses are widespread throughout the world, being isolated from 16 rodent species and 4 species of insectivores (41,42,43). Recently, Chinese scientists claimed that they have isolated Hantaan virus from domestic cats and wild rabbits in China (Song Gan, pers. common., 1989). Hantaviruses cause chronic asymptomatic infections in their reservoir hosts and in experimental animals, but Hantaan, Seoul and Puumala virus cause diverse

clinical symptoms in humans. It is quite likely that in the near future HFRS or HFRS-like diseases will be identified in many parts of the world where hantaviruses exist because of new knowledge and the availability of a simple serologic diagnostic test (38).

Seoul virus in Korea, Japan, China, and Sri Lanka causes HFRS but Seoul or Seoul-like viruses apparently do not cause disease in the Americas or in Africa. Further studies are needed to determine the human pathogenicity of Seoul or Seoul-like viruses from rodents in parts of the world where HFRS is not recognized in humans.

Presently, neutralization is the most sensitive and specific test for determining the infecting serotype. Our tests indicate that there are at least 6 hantavirus serotypes but we could not distinguish some strains of seoul virus isolated from house rats (Table 17). It may be that cross reactions between rat antisera against the viruses cause such confusion, therefore further studies with antisera produced in rabbits or guinea pigs are recommended to confirm the results. Recently, it was shown that Thottapalayam virus (13) from Indian shrew has a serologic relation with hantaviruses (C.H. Calisher, pers. comm., 1990) but it is too preliminary to determine the status of this virus.

The monoclonal antibodies produced with Hantaan virus were useful for differentiation of Hantaan, Maagi and Seoul viruses. The results clearly showed that there are 2 serologic subtypes of varieties of Seoul virus; and 4 strains did not react with monoclonal antibodies HC02-BZ08 and HC02-BD05 (Table 18). These results might be significant because, of these 4 Seoul virus strains, 3 (JTRN82/17, TR352, I/RN/82/3) were strains isolated from house rats and antibody to these 3 isolates had equal N antibody titers against Seoul and Hantaan viruses. However, by N tests Seoul virus Hubei/1, isolated from an HFRS patient in China, is different from these 3 Seoul-like viruses (JTRN/82/17, TR352, I/RN/82/3) isolated from house rats in Japan and Korea. The results of serologic relationships between hantaviruses using monoclonal antibody assays suggest that there are 7 serotypes as shown in Table 18.

Our results suggest that Nephropathia Epidemica (NE) in Finland is caused by either of 2 serotypes of Puumala virus: 1 is Puumala virus, the other virus has not yet been isolated. Evidences for this is that of 2 sera from NE patients that contained high FA antibody titers (4,096-16,384) against Puumala virus (Hallnas B and U.s.S.R. C1/18-20 strains). One serum (NE Fin 85-797)

showed high antibody titers (512-4,096) to Hantaan and Seoul viruses but the other serum (NE/Fin 85-802) had low antibody titers (32-256) to Hantaan and Seoul viruses (Table 18).

Table 19 shows the serotypes of hantaviruses isolated from HFRS patients and from other vertebrate hosts. We suggest that it is better to use serotype names for hantaviruses, rather than using the name of the host of origin to classify viruses. First, because more than one hantavirus serotype may be isolated from a single host and second, because many species of animals are reservoir hosts of the same hantaviruses.

The recent findings of hantaan virus antigens in tissues of birds in the U.S.S.R. (E.A. Tkachenko, pers. common., 1989) may have great impact on our understanding of both the epidemiology and the ecology of hantaviruses. It is possible that HFRS may become recognized as one of the important hemorrhagic diseases in many parts of the world in 1990s.

Table 19.
Serotypes of hantavirus isolated from HFRS patients and animals in the world.

Name of host	Serotypes of hantavirus isolated in Vero E6 cells
HFRS patient	Hantaan virus Seoul virus
Apodemus agrarius	Hantaan virus Maagi virus *
Urban rats	Seoul virus Seoul-like viruses *
Laboratory rats	Seoul virus Hantaan virus
Clethrionomys glareorus	Hantaan ** Puumala virus
Microtus pennsylvanicus	Prospect Hill virus
Golden hamster	Seoul virus
Bandicota indica	Seoul virus
Mus musculus	Leaky virus
Suncus murinus	Thottapalyam virus

* : Probable new serotype.
** : An isolate from USSR but origin is not clear.

CONCLUSION

1. There were 430 confirmed HFRS cases among 1,886 suspect hemorrhagic fever patients in Korea in 1989 and, 107 and 3 of them were ROK Army and US Army soldiers, respectively.
2. There were 358 cases of scrub typhus among 1,886 suspect hemorrhagic fever patients and 6 of them were ROK Army soldiers.
3. Large outbreaks of murine typhus and spotted fever were confirmed serologically and the nos. of patients were 359 and 83, respectively in 1989.
4. Outbreaks of murine typhus, scrub typhus and spotted fever were observed about the time of the epidemic season of HFRS in Korea.
5. Twenty-seven cases of leptospirosis occured in the spring and summer of 1989.
6. A case of HFRS patient was confirmed serologically in Jeju island in October 1989 for the first time. It has been known that Jeju island is free from HFRS for long tome. A few cases of murine typhus, scrub typhus and spotted fever were also documented in Jeju island.
7. Male is dominant groups in HFRS, murine typhus, spotted fever and leptospirosis but female is dominant group in scrub tyhpus.
8. The above mentioned hemorrhagic diseases occured in all age groups including childrens.
9. A simple and rapid serologic diagnostic test for HFRS was developed by a passive agglutination procedure using high density particles coated with purified Hantaan virus antigen. This method is more sensitive than IFAT and result could be read in 1 hr.
10. Serologic studies of 42 hantaviruses isolated from HFRS patients and from animals in the different parts of the world indicated that there are 6 or 7 serotypes.
11. In the 1990s, it is highly possible to identify HFRS and HFRS-like illnesses caused by hantaviruses in parts of the world where HFRS is not known because of the availability of simple and rapid serodiagnostic test.

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